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THE UTILITY OF SHORT-TERM TESTS FOR CARCINOGENS: A TECHNICAL REVIEW

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SUMMARY

The number of chemicals in use in industry has grown immensely in recent decades. Some of these chemicals are potential occupational carcinogens and need to be identified and controlled to prevent human cancer. Rapid, inexpensive and reliable short-term tests can be used in screening for potential occupational carcinogens and in setting priorities for control measures and further testing.

Short-term tests are based on a variety of biological and biochemical changes which have been associated with the actions of carcinogens. Over 100 short-term tests have been proposed for use in detecting carcinogens. Of these, the Ames test using Salmonella bacteria and certain tests using mammalian cells (mutation, chromosome aberration, DNA repair synthesis, sister-chromatid exchange, micronucleus test and cell transformation) are widely used.

These tests are usually performed on isolated cells which do not actually become cancerous. Thus, their interpretation in the context of human carcinogenicity must be made with caution. The statistical concepts of specificity, sensitivity and predictive value are introduced to aid this interpretation. Due to the inherent shortcomings of the tests and the small fraction of chemicals estimated to be carcinogenic, it is expected that many chemicals which are positive in short-term tests will prove to be noncarcinogenic: in contrast, almost all chemicals which are negative in the tests are likely to be noncarcinogenic. Short-term tests cannot at present replace long-term animal assays and are likely to be most useful in screening chemicals prior to animal testing. Further work is indicated in the validation of a few promising short-term tests and the statistical combination of their results to give a more accurate prediction of carcinogenicity.

INTRODUCTION

"We are living in a sea of chemicals that have not been tested for mutagenicity and carcinogenicity(1)." This sea of chemicals is being generated in part by the great synthetic capability of modern industry. By 1977, the computer registry of the American Chemical Society had recorded more than four million individual chemical substances. Moreover, the number of chemicals in the registry has been growing at an average rate of about 6,000 per

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week(2). Of this enormous population of chemicals, only about 6,000 have been tested for carcinogenicity, and about 1,000 have been reported to be carcinogenic(3). This initial group of tested compounds was selected because of a high probability of carcinogenicity and only a few per cent of the total population of chemicals may turn out to be carcinogenic. Nevertheless it seems clear that many more chemicals will be added to the list of potential human carcinogens(4).

The carcinogenicity of chemicals to humans can be evaluated directly only by the study of cases of cancer in humans and their associations with exposure to chemicals. Other methods such as animal testing and short-term testing are, at best, indirect measures of risk to humans.

The study of the patterns of disease and mortality in the human population and the factors that determine their distribution is called epidemiology. It is usually carried out after the fact: the incidence or prevalence of a particular cancer is found for a group of people who have had an exposure to a particular chemical. This "observed" cancer incidence or prevalence is then compared to the "expected" value for this cancer in the control population (usually a matched group of workers not exposed to the chemical of interest)(5). Occupational carcinogens detected in this way include the bladder carcinogen 2-naphthylamine(6) and the lung carcinogen asbestos(7).

Epidemiological studies are expensive and time consuming. Moreover, due to the complex exposure patterns in a workplace and problems with record keeping or recall, it is often difficult to pinpoint a specific chemical as an occupational carcinogen. Hence epidemiology usually designates areas of high risks or confirms suspected risks. It is rather insensitive in determining the carcinogenic risk of individual chemicals(8).

Animal study is an alternative for the evaluation and prediction of human carcinogens. The advantages of animal testing are that most human carcinogens have been shown to induce cancers in test animals, that animals can be treated with known doses of chemicals, and that cancers develop faster in small animals such as mice and rats than in humans.

Animal tests involve the treatment of experimental animals, usually rodents, by a relevant route of administration (orally, by inhalation, by implantation, or by skin painting) with the suspected chemical at dose levels which are high but not acutely toxic. The animals are kept under observation for most of their lifetimes (usually one to two years) and are carefully examined for tumors.

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Animal carcinogenicity tests also have some disadvantages. First, they are time consuming and costly: a test of a single chemical at two-dose levels in two species may require 3 years and cost $500,000US(9). Second, because of the costs, relatively small numbers of animals are examined (usually 300 or less). Consequently the tests are limited in sensitivity and weak carcinogens may not be detected. Third, laboratory animals are not just scaled-down human beings and may not respond to a chemical in the same way as humans(10). For these and other reasons the results of animal studies must be interpreted carefully and extrapolated to human beings with caution.

SHORT-TERM TESTS

In recent years, a large number of short-term tests have been developed as fast and inexpensive methods to detect potential human carcinogens. The tests use a variety of cells, tissues and chemical processes and detect changes which are associated with the development of cancer. Since the key events during carcinogenesis are not well understood, short-term tests are based on correlations between the actions of the test material and of known carcinogens in the same test. The test systems are still more "distant" to human beings than are animal tests and the results of short-term tests have to be interpreted with even more caution. Nevertheless, these tests have found widespread use as general screening tests for carcinogens.

Short-term tests may be classified into the following twelve groups according to the type of change detected:

1. **Tests Based on Mutagenesis.** These tests are based on the strong correlation between the carcinogenic (cancer-causing) and mutagenic (mutation-causing) activities of chemicals. Mutations are permanent inherited changes in the structure of the genetic material DNA: there is convincing evidence that certain specific mutations are among the initial events in the development of cancer(11).

One mutagenicity test, the Salmonella typhimurium/mammalian microsome test (Ames test)(1), is currently the most widely used short-term test: results of testing of over 12,000 chemicals have been published(12). This test is described in some detail to illustrate the features of short-term tests.

The basis of the test was the development of a series of strains of the bacteria Salmonella typhimurium. These strains have been mutated in various ways but in all cases they are unable to grow in the absence of the chemical histidine. If treatment of the bacteria with a carcinogen causes the specific mutation to be reversed then the bacteria can make their own

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histidine and can grow into a "colony" which can be detected readily. Mutations are scored by counting the number of colonies. In general, the higher the mutagenic potency of the test substance, the greater is the number of colonies. However the formation of colonies, and perhaps the detection of mutagenicity, can be prevented if the test substance is toxic enough to kill the bacteria.

The tester strains detect different classes of mutagens (e.g. TA1537, TA1538, TA97 and TA98 for frameshift mutagens and TA1535 and TA100 for base pair substitution mutagens)(13,14). Only certain strains may respond to a specific mutagen (and presumed carcinogen). From one to six strains of Salmonella typhimurium may be used in a single test. The accuracy of the test increases with the number of strains used, as does the cost.

The tester strains have been altered in other ways which increase their sensitivity to mutagens. Some strains contain a mutation (uvrB gene) rendering them unable to repair certain kinds of DNA damage(13). The permeability of the cell walls of Salmonella strains to chemicals has been increased by a mutation (deep rough rfa)(15). Finally, there is a further set of strains containing the drug resistance plasmid pKM 101(16).

One of the most important developments in short-term testing was the introduction of a liver extract ("microsomes" or "S-9"), usually from rats, containing enzymes which can convert many carcinogens to their active forms. This process is widely referred to as "metabolic activation." The use of this extract has greatly improved the success rate of the test(17) and is used in many other short-term tests. As a result of all these efforts the Ames test is able to detect very small amounts of a mutagen, usually micrograms (10^{-6} grams), and in some cases even nanograms (10^{-9} grams), of material.

Results of the Ames test in many laboratories over many years show an impressive correlation between mutagenicity and carcinogenicity. It detects most organic chemicals that are recognized human carcinogens except benzene and diethylstilbestrol(18). Most chemicals which are carcinogenic in animal tests have mutagenic activity which can be detected in the test(19). Similarly, the strains have generally failed to detect mutagenic activity for most chemicals which were noncarcinogenic in animal tests. The major failing of the test has been its lack of response to certain carcinogenic metals, several of which are carcinogenic in humans.

Mutagenesis tests have been developed using other bacterial strains such as Escherichia coli(20) and B. subtilis(21) and mammalian cells such as human fibroblasts, mouse cells and Chinese hamster cells(22,23). Mammalian cell tests offer the

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potential advantages of similarity to normal human cells, including transport and metabolism of chemicals and chromosomal structure. However, these tests are not as sensitive as was expected and may be considerably more difficult to perform. In practice the Ames test is almost universally used in screening programs with other mutation tests being used mainly for confirmation or elaboration of its results.

2. **Tests Based on Chromosomal Aberrations.** Chromosomes are thread-like structures in the cell nucleus and can be seen under a microscope. They are composed of long molecules of DNA which carry the cell's genetic information. Most carcinogens cause chromosomal aberrations (visible breaks and other damage); there is also a close association between chromosomal aberrations and the development of cancer(24). This provides the basis for the study of chromosomal aberrations in mammalian cells as a short-term test(11,24,25,26). Lymphocytes (one type of white blood cells from humans or test animals) are commonly used to assess the effects of chemicals on chromosomes. Cultured mammalian cells and mouse spermatogonial cells are also often used. A major advantage of this test is that it can be done using cells from exposed humans or animals (in vivo exposure). This test agrees fairly well with the Ames test and animal assays(24) but appears to give a somewhat higher frequency of false positive results: some (apparently) noncarcinogenic chemicals and other factors such as viral infection can increase the frequency of aberrations.

3. **Tests Based on Sister-Chromatid Exchange (SCE).** Sister chromatids are the two identical halves of a chromosome in a dividing cell. Sister-chromatid exchanges (SCEs) are interchanges of DNA between the two chromatids. Exchanges are thought to occur during repair of damaged DNA and SCEs are counted as a measure of DNA damage. They can be visualized readily in chromosomes from many types of mammalian cells(26,27,28). The SCE technique is relatively simple to perform and analyse and can be done using cells such as lymphocytes (white blood cells) from an exposed human and spermatogonial or bone marrow cells from a treated experimental animal. The test shows a greatly enhanced sensitivity to some carcinogens but not to others(29). Consequently SCE tests cannot yet be accepted as quantitative indicators of carcinogenic risk.

4. **Tests Based on Micronucleus Formation.** Micronuclei are fragments of chromosomes in the cell outside the nucleus. They result from breakage of chromosomes (DNA) or a malfunction of cell division(30,31). Micronuclei can be observed in blood or bone marrow cells from animals which have been exposed to certain carcinogens. However, it has a lower success rate in detecting carcinogens and noncarcinogens than the Ames test.

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5. **Tests Based on Repair of Damaged DNA.** Chemical damage to cellular DNA is repaired by the action of a system of enzymes. The measurement of this repair provides an indication of the ability of a chemical to damage DNA. The amount of DNA synthesis occurring during repair is usually measured from the uptake of a radioactive marker during the period immediately following exposure to the test chemical(32,33). Several DNA repair test systems are now available which use cells from bacteria, yeast and mammals, including humans(34). DNA repair tests detect effects of chemicals which may not be seen in the mutagenesis assays. Thus they are complementary to the mutagenesis tests and are often combined with mutagenesis tests as part of short-term test batteries. A major disadvantage of DNA repair tests is that they also measure DNA-damaging events which may not be associated with carcinogenesis.

6. **Tests Based on Cell Survival.** Cells deficient in repair of DNA are killed more easily by DNA-damaging agents than are normal (wild-type) bacteria. This difference offers a simple method for detecting DNA damage by measuring the survival of cells with varying DNA repair capacities. Bacterial strains deficient in excision repair(35) or recombination repair(36) and repair-deficient human Xeroderma pigmentosum cells(37) have been used. To date this method has been used more in research than in screening applications.

7. **Tests Based on Malignant Transformation.** Some types of cultured mammalian cells can be transformed into cancer cells by treatment with carcinogens: these cells will form tumors if injected into suitable animals. The transformed cells also have other altered properties which can be used as indicators in short-term tests(38,39,40). The most common indicators are the ability of the cells to form colonies similar to those characteristic of cancer cells. Two studies have shown prediction of carcinogenicity similar to that of the bacterial mutagenicity tests(41,42). Limitations to the current use of cell transformation as a test method include a high rate of "spontaneous" cell transformation, problems in defining criteria equating in vitro transformation with malignancy, and the technical skill needed to perform the assay.

8. **Tests Based on Accelerated Tumor Formation.** Various means of accelerating tumor formation to reduce the time needed for carcinogen testing have been proposed. These include the use of newborn animals(43), implanting embryonal tissues together with carcinogens into adult animals(44), and immuno-suppression(45). However, these accelerated carcinogen testing experiments still require several months or longer and sometimes acceleration does not occur.

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9. **Tests Based on Teratogenesis.** Many carcinogens are also teratogens (induce birth defects) and teratogenesis has been proposed as an indicator of potential carcinogens(46). A variety of carcinogens including derivatives of urethane and benz(a)anthracene have produced teratogenic changes in the offspring of treated female rodents(47). Studies to date indicate that teratogenicity is not highly correlated with carcinogenicity(46,48).

10. **Tests Based on Cytological Alterations.** Carcinogens cause changes in the structure and appearance of some cells and these changes can be used as a basis of screening. These include nucleolar changes(49) and microsome degranulation Rabin's test)(50). The size of the nucleolus was increased by thioacetamide but decreased by acridine orange and actinomycin D(51). Rabin's test predicted the carcinogenicity of aromatic amines well but was unsuccessful with other chemicals(52). These tests have not been extensively validated.

11. **Tests Based on Biological Changes.** These tests are based on changes in the growth or appearance of certain tissues following treatment with carcinogens. They include the sebaceous gland test(53,54) and implant test(41). These tests have not been well studied and appear to be used primarily in conjunction with animal carcinogen assays. They may also be useful as part of a battery of tests.

12. **Tests Based on Biochemical Reactions.** A number of chemical reactions have been identified which are characteristic of certain classes of chemical carcinogens. These include the tetrazolium reduction test(55), biphenyl test(56), iodine colour test(57) and acridine colour test(58). Since these tests are specific for certain kinds of carcinogens they are considered to be unsuitable for general use in screening programs(41).

From this brief overview of short-term tests it is clear that a large number of short-term tests are available for carcinogen detection. In fact Hollstein and McCann(18) have identified over 100 short-term assays. Among these tests, the Ames test is by far the most popular test and is usually adopted for routine screening in many laboratories. Other tests that are commonly used include mutation of mammalian cells, chromosomal aberration, DNA repair synthesis, sister-chromatid exchange, micronucleus formation, and cell transformation.

Most of the short-term tests which are at present widely used for the detection of potential carcinogens are assays for the mutagenic activity of chemicals or of their ability to damage DNA(11). A good correlation between chemicals causing cancer and those causing mutation has been demonstrated(16,59,60), which suggests that a common molecular event may be shared by the two processes. However short-term tests can mimic only a small

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part of the total complex process of carcinogenesis(61). Thus, their results must be interpreted with care if any reference to carcinogenicity is to be made.

THE VALIDITY OF SHORT-TERM TESTS

In order to appreciate the role of short-term tests as predictors for the carcinogenicity of chemicals the following terms are defined:

The VALIDITY of a test is defined as the accuracy with which a test measures what it is intended to measure. A carcinogen-screening test should provide a preliminary indication of which chemicals are carcinogenic and which are not. This is referred to as the validity of the test. Validity has two components: sensitivity and specificity. SENSITIVITY is defined as the ability to identify correctly the substances that produce cancer(62,63). SPECIFICITY is defined as the ability to identify correctly those which do not produce cancer.

Because short-term tests do not have the production of tumours in animals as an end point, such tests have to be validated using a number of known carcinogens and noncarcinogens. The extent to which the screening results agree with those derived from human data and animal tests provides a measure of sensitivity and specificity. An ideal screening test would be 100 per cent sensitive and 100 per cent specific. In practice this does not occur; sensitivity and specificity are usually inversely related. That is, one usually achieves high sensi- tivity at the expense of low specificity.

Many studies have been carried out to evaluate the success rates of short-term tests in identifying carcinogens and noncarcinogens (Table, p. 14). The Salmonella/Ames test is the best studied and most extensively validated short-term test and can exemplify the use of statistical concepts. So far the largest validation study on the Ames Test was carried out by McCann and co-workers who applied the test to about 300 chemicals(19). Among 175 carcinogens tested, 157 (90%) gave positive results and 87% (94/108) of the noncarcinogens gave negative results.

In this study the screening level was set at 0.01 revertants per nanomole (nmol, 10^{-9} mole), i.e. any test result greater than this screening level was considered to be positive. A sensitivity of 90% and a specificity of 87% were obtained. Had the screening level been set higher, say 0.1 revertants per nmol (this is equivalent to treating all weak mutagens as nonmutagens) the sensitivity would have become 81% (141/175) and specificity would have been 90% (97/108).

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When carcinogens are labelled negative, the test results are referred to as false negatives. When noncarcinogens are called positive, the test results are false positives. Naturally, a sensitive test will create few false negatives and a specific test will give few false positives.

An important issue in carcinogenicity testing is the ability of tests to predict carcinogenicity(64). Sensitivity and specificity are measures of the accuracy with which a test identifies carcinogens and noncarcinogens. They are not measures of the accuracy to predict carcinogenicity and noncarcinogenicity. At the time a test is first evaluated, it is performed on a group of carcinogens and noncarcinogens and the indices of sensitivity and specificity are calculated. If the results seem sufficiently encouraging, the test might be accepted into general laboratory usage.

While the original investigators started with a group of substances for which carcinogenicity had already been confirmed, the investigators who later use the screening test must start with substances whose carcinogenic properties are unknown. The purpose of the test is to predict whether the substance can be carcinogenic or not. In receiving the result of the test, an investigator therefore wants to know its predictive accuracy, not its sensitivity or specificity. If the test result is positive, what is the probability that the substance is a carcinogen? If the test result is negative, what is the probability that the substance is a noncarcinogen? To answer these two questions, a different set of indices, the predictive values, have to be defined.

PREDICTIVE VALUE OF A POSITIVE TEST (PV+) is defined(65,62) as the probability that a substance with a positive test result is a carcinogen. It is estimated by the proportion of carcinogens among the substances which are positive to the test. PREDICTIVE VALUE OF A NEGATIVE TEST (PV-) is defined as the probability that a substance with a negative test result is a noncarcinogen. It is estimated by the proportion of noncarcinogens among the substances which are negative to the test.

Continuing the example of the validation study of McCann et al. (1975b), the Ames test has the following properties:

Sensitivity	= 90% (157/175)
Specificity	= 87% (94/108)
False positive rate	= 13% (14/108)
False negative rate	= 10% (18/175)
PV+	= 92% (157/(157+14))
PV-	= 84% (94/(94+18))
Proportion of carcinogens	= 62% (175/(175+108))

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In this validation study, the group of test substances consists of 62% carcinogens and 38% noncarcinogens. In a real-life situation, however, the proportion of carcinogens (or the probability that a test substance selected at random might be a carcinogen) is likely to be much lower, and will vary according to the class of chemicals to which the test substance belongs. Had the validation study been applied to a group of substances with a proportion of carcinogens of 1%, with the same sensitivity and specificity the PV+ would have dropped drastically to 6.5% and the PV- increased to 99.9%. It would be very disappointing to learn that given that a substance has a positive result in the Ames test, the probability that it is a carcinogen is only 6.5%(59,66,67,68). The low value of PV+ is due to the large number of noncarcinogens (in this case 99%) which give rise to a large total number of false positives in spite of a low false positive rate. If the proportion of carcinogens is assumed to be 10% in this example then the PV+ increases to about 43% and the PV- drops slightly to 98.7%.

ORIGINS OF FALSE-POSITIVE AND FALSE-NEGATIVE RESULTS

There is little prospect that any short-term test can be improved to give both 100% sensitivity and specificity. There may always be a finite level of false-positive and false-negative results. Where do the false positives and false negatives come from? Taking the Ames test as an example, the false positives and false negatives may arise from several sources(10,52,69,70,71):

False positives. "Noncarcinogens" positive in the Ames test may be due to the following reasons:

1. Inadequacies in animal carcinogenicity tests. For example, the food additive furyl furamide gave negative results in two animal tests but was eventually shown to be a weak carcinogen: the "noncarcinogen" was in fact a carcinogen. Other compounds such as sodium azide can give equivocal results in animal assays. If a true carcinogen is assumed noncarcinogenic on the basis of an animal assay then a positive short-term test result will be taken as a false positive.

2. Specific bacterial activation of chemicals. For example, nitro-compounds are activated to mutagens by nitro-reductases in the bacteria. These enzymes are not common in mammalian cells and treated animals would not likely develop tumors.

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3. Oversensitivity of some tester strains due to a defective repair system. For example, they could be reverted by chance exposure to ultraviolet light and give false positive test results.

4. Mutagenic impurities. For example, samples of 5-hydroxy-2-acetylaminofluorene have been shown to be mutagenic because of the presence of an impurity. Thus it is important to test the pure compound, the technical compound and common impurities.

5. Compounds enhancing the growth of cells in the assays. Compounds which stimulate cell growth may increase the chance that spontaneous mutations (false positives) can occur.

False negatives. Carcinogens negative in the Ames test may be attributed to the following reasons:

1. Inadequacies in the in vitro metabolic activation system. If the substance is left to stay for a longer period with the liver cell preparation, there is a higher chance of being activated. For example, the mutagenicity of dimethylnitrosamine can be detected by a preincubation at high enzyme concentrations. Moreover, the isolated liver extract may be an inadequate model for the complex biochemical processes involved in the detoxication and activation of carcinogens in the intact body. For example, the natural material cycasin is converted to methylazoxymethanol (which is carcinogenic) by microorganisms in the intestine and not by the liver.

2. Bacterial toxicity of the compound which may mask the mutagenic effect. Certain compounds may be much more toxic to bacteria than to animals. For example, some chlorinated pesticides can be detected as mutagens only in a narrow range of doses just below the concentrations which kill the bacteria.

3. Lack of tester strains sensitive to particular mutagenic effects. For example, the new tester strain TA94 can detect mitomycin C and malondialdehyde which were not detected by other strains.

4. Mutagens which are unique to animal cells and cannot be detected using bacterial tests. An example is griseofulvin which interferes with microtubule formation. Since microtubules are absent in bacterial cells, griseofulvin does not affect the bacteria.

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5. Carcinogens using a mechanism other than somatic mutation. For example, hormones may cause cancer in animals by affecting cell growth.

6. Chemical reactions and pH effects in the plate assay. Certain chemicals may be mutagenic only under special conditions which are not duplicated in the standard assay. For example, it can be difficult to test chemicals which react with chemical components of the assay system, which form insoluble compounds, or which are inactive at the pH used in the assay. If these problems are anticipated then a specially modified assay may be required.

No test is perfect and there is an upper limit of accuracy which any test can achieve. It could be counterproductive to develop a test, find that it is imperfect, drop it, and try to develop another test.

There are two courses available at the present stage. First, the relatively more accurate short-term tests that are available can be improved, standardised and more fully validated so that the accuracy coefficients are better estimated. So far the sensitivity of the Ames test in various validation studies ranges from 63% to 95% (Table, p. 14), which is obviously inadequate. Secondly, a number of screening tests, say five, may be grouped to form a test battery to replace the single-test approach. This is particularly important since there may be many mechanisms for the actions of carcinogens and a battery of tests may be needed to detect these different effects. For example, positive results in 3 tests and negative results in 2 tests are more informative and meaningful than a negative or positive result in a single test. Statistical treatments to combine results from various short-term tests are currently under investigation.

At the present stage of development of short-term carcinogenicity tests, it appears that no test or battery of tests can replace long-term animal experiments in determining the carcinogenicity of a substance. They serve primarily as prescreens to select substances which can be submitted with priority for bioassay in experimental animals.

Few people would disagree with attempts to remove a recognizable source of potential hazard, such as the presence of a potent animal carcinogen in the working environment, but many would question whether abrupt action should be taken on finding that a glue contained chemicals mutagenic to Salmonella typhimurium. There is still a long way to go with short-term carcinogenicity testing.

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SUCCESS RATES OF SOME SHORT-TERM TESTS

Numbers in brackets indicate the number of carcinogens or noncarcinogens tested

Test System	Sensitivity	Specificity	Reference
Salmonella/Ames	90% (175)	87% (108)	19
Sammonella/Ames	63% (27)	79% (33)	72
Salmonella/Ames	72% (65)	78% (26)	73
Salmonella/Ames	91% (58)	93% (62)	41
Salmonella/Ames	92% (98)	---	60
Salmonella/Ames	65% (36)	81% (16)	30
Salmonella/Ames	71% (51)	---	18
Salmonella/Ames	95% (22)	44% (27)	71
Salmonella/Ames	76% (82)	57% (7)	74
E. coli pol A mutagenesis	69% (58)	75% (24)	73
E. coli pol A mutagenesis	57% (44)	---	18
Cell transformation	91% (58)	97% (62)	41
Cell transformation	81% (26)	---	18
Rabin's test	71% (58)	71% (62)	41
Subcutaneous implants	37% (58)	95% (62)	41
Sebaceous gland suppression	67% (58)	64% (62)	41
Tetrazolium reduction	40% (58)	73% (62)	41
Micronucleus	43% (36)	81% (16)	30
Micronucleus	68% (22)	---	18
Micronucleus	58% (59)	82% (22)	75
Abnormal sperm	65% (36)	62% (16)	30
Abnormal sperm	68% (19)	---	18
Unscheduled DNA synthesis	55% (22)	---	18
DNA repair	94% (16)	---	18
Dominant lethal	32% (25)	---	18
Sister-chromatid exchange in vitro	92% (26)	---	18
Sister-chromatid exchange in vivo	85% (13)	---	18
Chromosome aberration in vitro	48% (27)	73% (33)	72
Chromosome aberration	78% (27)	---	18
Chromosome aberration	82% (17)	---	18

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